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Integrated processes for expansion and differentiation of human pluripotent stem cells in suspended microcarriers cultures

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ABSTRACT

Current methods for human pluripotent stem cells (hPSC) expansion and differentiation can be limited in scalability and costly (due to their labor intensive nature). This can limit their use in cell therapy, drug screening and toxicity assays. One of the approaches that can overcome these limitations is microcarrier (MC) based cultures in which cells are expanded as cell/MC aggregates and then directly differentiated as embryoid bodies (EBs) in the same agitated reactor. This integrated process can be scaled up and eliminate the need for some culture manipulation used in common monolayer and EBs cultures. This review describes the principles of such microcarriers based integrated hPSC expansion and differentiation process, and parameters that can affect its efficiency (such as MC type and extracellular matrix proteins coatings, cell/MC aggregates size, and agitation). Finally examples of integrated process for generation cardiomyocytes (CM) and neural progenitor cells (NPC) as well as challenges to be solved are described.

Contents

1. Introduction

Human pluripotent stem cells (hPSC), including human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC), have the ability to expand and differentiate to multiple cell types, thus are valuable tools for use in cell therapy, drug screening, toxicity assays and disease modeling [1]. For these

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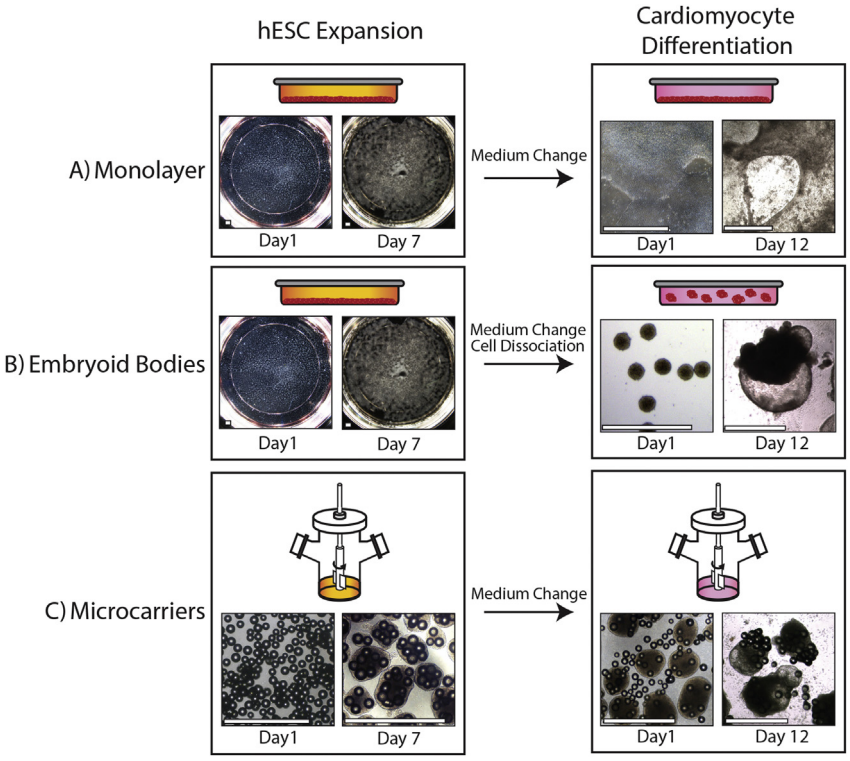

Fig. 1. Current methods for hPSC expansion and differentiation. (A) 2D monolayer process which rely on 2D planar culture. Cell expansion and differentiation can be carried out in a single unit. However, it requires handling of multiple units for scale-up. (B) EBs based cultures which rely on planar cell expansion followed by formation of cell aggregates (EBs) for differentiation. (C) MC based integrated bioprocesses which allow hPSC expansion and differentiation to be carried out as a single unit operation in the same bioreactor. The advantages of this approach are: high cell yields; controlled aggregate size; time- and cost-saving, and has the potential to comply with a defined GMP bioprocess system. Scale bar = 1 mm.

applications efficient large scale and sometimes Good Manufacturing Practice (GMP) compatible methods for expansion and differentiation should be developed [2].

However, current cultivation methods can be inefficient, labor intensive and expensive [3]. One of the approaches to overcome these difficulties is to develop an integrated cell expansion and differentiation process in one stirred reactor using microcarriers (MC) based cultures. This review will describe recent progress and challenges in developing such systems with a goal to develop a scalable, xeno-free, chemically defined, and cost effective culture system clinical-grade expansion and differentiation of hPSC that comply with the requirements of current GMP.

2. Expansion of hPSC: two-dimensional (2D) cultures *vs* three-dimensional (3D) MC cultures

Current 2D monolayer culture methods for hPSC expansion have shortcomings such as limited available area for cell growth (Fig. 1A), labor-intensive operation, and the lack of continuous monitoring and control of the culture environment [4]. The MC system, in which cells are growing as aggregates of cells and MC (Fig. 1C) suspended in stirred reactors, can achieve higher cell yields [3], better control of culture parameters as well as the ability to scale up volumetrically.

Our group [5–9] and others [10–12] have reported that several hESC and hiPSC can be expanded on a variety of MCs coated with different extracellular matrix (ECM) proteins in serum or serum free media achieving higher cell yields, 2.4–4.3 fold more cells than those on 2D culture plates [7–9]. The expanded cells maintain their pluripotency, normal karyotype as well as the ability to differentiate into the three germ layers [9,13]. Moreover since these cultures are maintained as cell/MC aggregates it can be further differentiated directly (without cell dissociation) into a variety of cell types [7,8,13].

3. Differentiation of hPSC: 2D cultures and EBs *vs* 3D MC cultures

The general differentiation platforms reported in the literature uses 2D monolayer culture or 3D embryoid bodies (EBs) culturing systems (Fig. 1A and B). In monolayer cultures, scaling up is limited by the available surface area and control of differentiation is problematic [14]. The 3D EBs systems, which rely on cell aggregation, have the potential of volumetric scaling up since they are suspended in the culture. However, the multiple steps of cell dissociation or manual cutting of the monolayer cultures to generate cell aggregates (Fig. 1B) can cause cell losses, forms large variability in size (which affect differentiation efficiency), are laborious and is difficult to scale up [15].

On the other hand hPSC propagated in stirred-suspension 3D MC cultures have been reported to generate even sized cell MC aggregates (about 300–400 μm [7,8]) which can be used as EBs for direct differentiation into a variety of differentiated cells without the need for cell dissociation and exhibit the potential to scale up and implement culture environmental control. Differentiation to definitive endoderm [16], cardiomyocytes [17–20], neural progenitor cells [21], hepatocytes [22], and hematopoietic cells [23] in the MC cultures have been reported.

In general, differentiation efficiency in the MC systems is higher than in EBs cultures. For example, in MC based cardiomyocytes differentiation 2–3-fold higher yield is reported as compared to the EBs method [20,24].

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